February 13, 2001, with Dr. Stamler and the undersigned attorney participating. Reconsideration and further examination of the application are requested.

Also presented as a section of this paper is an Interview Summary, which is a brief account of the interview of Applicant Jonathan S. Stamler, M.D. and the undersigned attorney Carol A. Egner with the Examiner on February 13, 2001.

Also being filed at this time is a separate paper entitled "Supplemental Amendment," which addresses the issue in Item 10 of the Office Action dated 12 January 2001.

Please amend the application as follows:

In the Specification

Please replace the paragraph at page 76, line 28 through page 77, line 29 with the following paragraph:

We proposed that the degree of hydrogen bonding between bound oxygen and the distal histidine was critical in determining the degree of oxidation of hemoglobin by nitric oxide. Therefore, we examined the degree of oxidation of hemoglobin by nitric oxide in a variety of buffers. 5 ml of phosphate buffer containing 300 µM Hemoglobin A (~95% oxyHb) was placed in a 15 ml vial. Nitric oxide was added from a stock solution, 2 mM, stored under nitrogen. Immediately after nitric oxide addition, the absorbance at 630 nm was measured, and the concentration of oxidized (metHb) was plotted, using 4.4 as the extinction coefficient for metHb at 630 nm. Experiments were performed in 1 M, 100 mM, and 10 mM sodium phosphate buffer (pH 7.4). The data in Figure 19 show higher oxidized hemoglobin formation in 1M phosphate, which is indicative of a higher effective substrate concentration, as would be predicted by phosphate destabilization of the hydrogen bond between iron bound oxygen and the distal histidine. At the lowest concentrations of nitric oxide added, S-nitrosothiol was formed under all conditions (approximately 5 µM). Additions of nitric oxide at concentrations of 30 µM or greater resulted in the additional formation of nitrite. The presence of 200 mM borate within the buffer reduced oxidized hemoglobin and nitrite formation, whilst the presence of either 200 mM

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acetate or chloride increased the formation of oxidized hemoglobin and nitrite. Addition of nitric oxide to hemoglobin in 10 mM phosphate buffer at a ratio of less than 1:30 (NO:Hemoglobin A) resulted in the formation of S-nitrosothiol without production of oxidized hemoglobin. S-nitrosothiol formation was optimized by adding the nitric oxide to hemoglobin in 10 mM phosphate, 200 mM borate, pH 7.4. Therefore, the balance between oxidation and nitrosothiol formation is dependent upon the ratio of nitric oxide to hemoglobin and the buffer environment.

Amend Claims 16, 67, 68, 70 and 71. Amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (pages i-ii).

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- 16. (Twice Amended) A method for potentiating delivery of NO to tissues in a mammal, comprising administering to the mammal an effective amount of a mixture of a low molecular weight thiol and hemoglobin or nitrosated hemoglobin.
- 67. (Amended) A composition comprising S-nitrosylated oxyhemoglobin.
- (Amended) A method for making a composition comprising S-nitrosylated oxyhemoglobin, comprising incubating excess nitrosating agent with purified hemoglobin in the presence of oxygen at pH 7.4 to 9.2.
 - 70. (Amended) A composition comprising S-nitrosylated deoxyhemoglobin.

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71. (Amended) A method for making a composition comprising S-nitrosylated deoxyhemoglobin, comprising incubating excess nitrosating agent with purified hemoglobin in the absence of oxygen at pH 7.4 to 9.2.